

Effect of Different Levels of Inorganic Chromium on Performance and Immunity of Broiler Chicks

¹Farshid Kheiri and ²Majid Toghyani

¹Department of Animal Science, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

²Department of Animal Science, Islamic Azad University, Khorasgan Branch, Esfahan, Iran

Abstract: This experiment was conducted to investigate the effect of different levels of chromium chloride on performance and immune responses of broiler chicks. Three hundred and one days old broilers (Ross 308) were allocated to five treatments with four replicates in a completely randomized design. Treatments supplemented with 0 (control), 400, 800, 1200 or 1600 $\mu\text{g kg}^{-1}$ chromium in the form of chromium chloride. Body weight, feed intake and feed conversion were measured in different periods. At 18 and 28 days serum antibody titres against newcastle and influenza virus were determined. At 42 days, heterophil to lymphocyte and albumin to globulin ratios were measured. Lymphoid organs and carcass traits were measured at 42 days. Body weight improved significantly ($p < 0.05$) in broilers fed 1600 $\mu\text{g kg}^{-1}$ supplemental chromium. Feed intake, feed conversion, lymphoid organs and carcass traits (except of carcass yield) were not affected by supplemental chromium ($p > 0.05$). Antibody titres against newcastle virus in broilers received 1600 $\mu\text{g kg}^{-1}$ chromium supplementation were elevated ($p < 0.05$). Heterophil to lymphocyte and albumin to globulin ratios were not affected by dietary chromium ($p > 0.05$). The results of this experiment indicated that chromium supplementation improved body weight, carcass yield and antibody titre against Newcastle virus in broiler chicks.

Key words: Broiler, chromium chloride, performance, carcass trait, immunity, body weight

INTRODUCTION

Trivalent Chromium (Cr^{+3}) is a well known essential trace element for human and animals (Schwartz and Mertz, 1959). Cr is a component of an oligopeptide low molecular-weight Cr-binding substance, chromodulin, functioning as a part of the insulin signaling auto-amplification mechanism (Vincent, 2000). This stimulation of insulin action, which is directly proportional to the Cr content of the chromodulin, occurs without changing the concentration of insulin required for half-maximal activity (Vincent, 2000). This element is also involved in carbohydrate, lipid, protein and nucleic acid metabolic function (Ohba *et al.*, 1986; McCarty, 1991).

Dietary Cr supplementation has been shown to positively affect growth rate and feed efficiency in growing poultry (Jackson *et al.*, 2008; Samanta *et al.*, 2008; Uyanik *et al.*, 2002; Sahin *et al.*, 2002; Lien *et al.*, 1999; Amatya *et al.*, 2004). Improvements in immune responses have been observed when Cr were supplemented to broilers (Toghyani *et al.*, 2007; Luo *et al.*, 1999), stressed feeder calves (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993) and

dairy cows (Burton *et al.*, 1993). However, recommendations regarding the exact dietary inclusion level of Cr^{3+} in diets of livestock, including poultry are yet to be established (NRC, 1994) and thus, need further research.

The present experiment was conducted with broiler chickens receiving either no Cr supplementation or different levels of Cr from Cr chloride. The objective of this study was to assess the effects of these supplemental Cr on performance and immune responses of broiler chicks.

MATERIALS AND METHODS

Birds and diet: Three hundred days old Ross 308 broiler chicks were purchased from a local hatchery. Immediately after arrival, water containing glucose and electrolytes was offered to the birds, followed by weighing and placing of the birds on litter in pens measuring 1.2×1.2 m. A single pen constituted a replicate and the pens were the experimental units in the present investigation. The birds were divided according to their body weight into five designated treatment groups, with each group consisting of four replicates ($n = 15$ per replicate).

Birds were fed *ad libitum* with a corn-soybean meal basal diet for starter from 0-10 days (ME: 2825 kcal kg⁻¹, CP: 22.28%), grower from 11-28 days (ME: 3000 kcal kg⁻¹, CP: 20.5%) and finisher from 29-42 days (ME: 3050 Kcal kg⁻¹, CP: 18.6%). The basal diets were formulated to meet the nutrient requirements of Ross 308 broiler chicks.

The birds received dietary Cr³⁺ supplementation for 42 days (days 1-42) and the dietary treatments consisted of feeding the birds a basal diet (control), the basal diet supplemented with 400, 800, 1200 and 1600 µg Cr kg⁻¹. Chromium chloride (manufactured by Merck company, Germany) containing 18.5% Cr was the source of the supplemental Cr³⁺. The unsupplemented basal starter, grower and finisher diets contained 3.21, 3.82 and 4.21 mg kg⁻¹ Cr, respectively, as measured by atomic absorption spectrometer with a graphite furnace (Perkin-Elmer, AAnalyst 600, USA).

Performance and carcass traits: Body weight, feed intake and feed conversion ratio were measured at 11, 28 and 42 days of age. At 42 days of age three birds were chosen randomly from each replicate, slaughtered and abdominal fat pad, liver and pancreas were removed, weighed and expressed as a percentage of live body weight.

Lymphoid organs: Three birds from each replicate were slaughtered on day 42 and lymphoid organs such as thymus, spleen and bursa of Fabricius were collected, weighed and expressed as a percentage of live body weight.

Immune responses: All birds were intramuscularly immunized with killed vaccine of Newcastle and Avian Influenza (H9 N2) viruses at age of 8 days. On days 18 and 28, blood samples were collected from the wing vein of three birds per replicate and serum antibody titer against Newcastle and Influenza viruses were determined by Haemagglutination Inhibition (HI) test and were expressed as the logarithm base 2.

At 42 days of age, blood samples of three chicks from each replicate were obtained from the wing vein using heparin as anticoagulant. Blood smears were prepared

using May-Grunwald-Giemsa stain and heterophil to lymphocyte ratios were based on a total of 100 cells (Gross and Siegel, 1983).

Serum protein fractions were separated by electrophoresis (150 V/25 min) in cellulose polyacetate. Five fractions were obtained (albumin, α₁, α₂, β and γ globulin) and albumin to globulin ratio was calculated.

Statistical analysis: The experiment data were analyzed by analysis of variance procedures appropriate for a completely randomized design using the GLM procedures of SAS Institute (1997). Significant differences (p<0.05) among treatment means were determined using Duncan's new multiple range test.

RESULTS

The effects of Cr supplementation on performance of broiler chicks are shown in Table 1. Supplement of 1600 µg kg⁻¹ Cr to broiler diets significantly increased weight gain (p<0.05) and body weight of broilers at 42 days of age. Feed consumption and feed conversion ratio of broilers was not affected by different levels of supplemental Cr (p>0.05).

Table 2 shows the effects of Cr supplementation on carcass traits of broiler chicks. Carcass yield and abdominal fat were not significantly affected by supplemental chromium (p>0.05). Carcass yield of broilers fed 1600 µg kg⁻¹ Cr tended to increased and abdominal fat to decreased in broiler fed 1200 and 1600 µg kg⁻¹ Cr (p>0.05). Percentage of liver and pancreas to live body weight were not affected by different levels of Cr.

Chromium supplementation had not significant (p>0.05) effect on lymphoid organs weight (Table 3). However, spleen weight of broiler chicks fed 1600 µg kg⁻¹ Cr were greater than other groups and control.

The effects of Cr supplementation on antibody titers against Newcastle and Influenza virus are shown in Table 4. Antibody titers against Newcastle virus at 18 days were significantly affected by supplemental Cr and broilers fed 1600 µg kg⁻¹ Cr had higher antibody titers in comparison with control (p<0.05). Cr supplementation not significantly (p>0.05) tended to elevate antibody titers against Influenza virus at 18 days.

Table 1: Effect of different levels of chromium chloride on performance of broiler chicks

Parameters	Chromium chloride (µg kg ⁻¹)					SEM
	Control	400	800	1200	1600	
Final live weight (g)	2091.30 ^b	2096.70 ^b	2106.00 ^b	2097.20 ^b	2156.70 ^a	15.200
Weight gain (g day ⁻¹)	50.00 ^b	50.20 ^b	50.40 ^b	50.10 ^b	51.60 ^a	0.420
Feed intake (g/bird/day)	85.30	85.40	85.10	85.50	85.40	1.540
Feed conversion ratio (feed: gain)	1.71	1.70	1.69	1.71	1.65	0.189

^{a,b}Means within the same row without common superscripts differ significantly (p<0.05)

Table 2: Effect of different levels of chromium chloride on carcass traits of broiler chicks at 42 days of age

Carcass traits*	Chromium chloride ($\mu\text{g kg}^{-1}$)					SEM
	Control	400	800	1200	1600	
Carcass	68.700 ^b	69.700 ^b	70.000 ^b	69.900 ^b	71.500 ^a	0.682
Abdominal fat	1.480 ^a	1.560 ^a	1.590 ^a	1.340 ^b	1.350 ^b	0.981
Liver	2.120	2.180	1.960	1.930	2.160	0.451
Pancreas	0.207	0.206	0.206	0.198	0.201	

Table 3: Effect of different levels of chromium chloride on lymphoid organs of broilers at 42 days of age

Lymphoid organ*	Chromium chloride ($\mu\text{g kg}^{-1}$)					SEM
	Control	400	800	1200	1600	
Bursa of fabricius	0.054	0.059	0.062	0.063	0.061	0.012
Spleen	0.122	0.112	0.119	0.124	0.182	0.074
Thymus	0.296	0.184	0.166	0.287	0.191	0.253

^{a,b}Means within the same row without common superscripts differ significantly ($p < 0.05$), *: Percentage of live weight

Table 4: Effect of different levels of chromium chloride on antibody titers against Newcastle and Influenza virus at different ages

Chromium levels ($\mu\text{g kg}^{-1}$)	Newcastle (\log_2 HI titer)		Influenza (\log_2 HI titer)	
	18 days	28 days	18 days	28 days
	Control	1.53 ^b	1.33	3
400	2.66 ^{ab}	1.53	4.33	3.53
800	1.83 ^{ab}	1.66	3.83	3.16
1200	2.66 ^{ab}	2.16	3.53	2.16
1600	2.83 ^a	1.33	3.83	2.66
SE	0.37	0.35	0.71	0.45

Table 5: Effect of different levels of chromium chloride on Heterophil to Lymphocyte ratio (H/L) and Albumin to Globulin ratio (A/G) at 42 days of age

Chromium levels ($\mu\text{g kg}^{-1}$)	H/L	A/G
Control	0.650	0.590
400	0.630	0.500
800	0.720	0.530
1200	0.590	0.640
1600	0.430	0.680
SEM	0.082	0.055

^{a,b}Means in the same column with no common superscripts differ significantly ($p < 0.05$)

Table 5 shows the effects of Cr supplementation on Heterophil to Lymphocyte ratio (H/L) and Albumin to Globulin ratio (A/G) at 42 days of age ($p > 0.05$). Cr supplementation had not significant effect on H/L and A/G. However, H/L tended to decrease in broiler chicks received 1600 $\mu\text{g kg}^{-1}$ Cr.

DISCUSSION

The present study revealed that Cr supplementation particularly at level of 1600 $\mu\text{g kg}^{-1}$ improved the performance of the broiler chickens in terms of live-weight gain. This is in agreement with the observations of Nam *et al.* (1995) and Amatya *et al.* (2004) reported performance of broilers received Cr chloride was improved. Lien *et al.* (1999) reported that 1600 and 3200 $\mu\text{g kg}^{-1}$ Cr picolinate supplementation in a broiler

diets improved live weight gain. Kim *et al.* (1996) also, observed that 1600 $\mu\text{g kg}^{-1}$ Cr picolinate supplementation increased the weight gain in broilers. Toghyani *et al.* (2006) reported body weight of broiler chicks received 1500 $\mu\text{g kg}^{-1}$ Cr in heat stress condition improved but feed conversion were not affected. Improvement in growth performance maybe related to better metabolizability of nutrients by Cr supplementation (Ahmed *et al.*, 2005).

The results of this study indicate that Cr supplementation increased carcass yield and tended to decrease abdominal fat (Table 2). In accordance with these results, increasing carcass yield and decreasing abdominal fat content in broilers has been reported for diets supplemented with Cr (Sahin *et al.*, 2002; Debski *et al.*, 2004). Samanta *et al.* (2008) reported dietary Cr supplementation improved carcass yield of broilers. In broiler chickens, supplementation of 100-400 $\mu\text{g kg}^{-1}$ Cr increased carcass protein with a simultaneous reduction in the fat content of the carcass (Kim *et al.*, 1996). Accretion of protein in the carcass was perhaps due to the potentiation of insulin action under the influence of Cr that might in turn have promoted the tissue uptake of protein. On the other hand, Cr was found to exert inhibitory effects on *in vitro* lipogenic activity in chick adipose tissue (Kim *et al.*, 1996).

Dietary Cr supplementation increased antibody titers against Newcastle and Influenza virus (Table 3). The role of Cr in the immune responses of mammals and chicken is well established (Burton *et al.*, 1993; Lee *et al.*, 2003). It has also been reported that chromium modulates the immune response through its effect on cytokine release (Wang *et al.*, 1996). However, the results obtained so far had been inconsistent, varying with each experiment.

Elevated antibody titer against Newcastle disease was reported in broiler chicks with supplement of 2 or 10 mg kg^{-1} Cr, either in the form of CrCl_3 or yeast

(Guo *et al.*, 1999). Lee *et al.* (2003) reported antibody titer against infectious bronchitis was improved in broiler chicks fed 400 µg kg⁻¹ Cr picolinate. Bhagat *et al.* (2008) reported supplementation of chromium at appropriate dose might be helpful to enhance the IFN- γ mRNA expression in response to Newcastle disease vaccine. Reports on swine, bovine and other species suggested variable immune response by the chromium. Reports exist of suppressed (Khangarot *et al.*, 1999) or enhanced (Burton *et al.*, 1993) or no significant effect of Cr on immune status (Van De Ligt *et al.*, 2002). Disparity amongst various reports could be due to the differences in chromium form, dosage, route or species.

CONCLUSION

The results of this study showed that dietary supplementation of Cr chloride, at level of 1600 µg kg⁻¹ improved performance, carcass yield and some immune responses in broiler chicks.

REFERENCES

- Ahmed, N., S. Haldar, M.C. Pakhira and T.K. Ghosh, 2005. Growth performances, nutrient utilization and carcass traits in broiler chickens fed with a normal and a low energy diet supplemented with inorganic chromium (as chromium chloride hexahydrate) and a combination of inorganic chromium and ascorbic acid. *J. Agric. Sci.*, 143: 427-439. DOI: 10.1017/s0021859605005617.
- Amatya, J.L., S. Haldar and T.K. Ghosh, 2004. Effects of chromium supplementation from inorganic and organic sources on nutrient utilization, mineral metabolism and meat quality in broiler chickens exposed to natural heat stress. *Anim. Sci.*, 79: 241-253. ISI: 000223995000006.
- Bhagat, J., K.A. Ahmed, P. Tyagi, M. Saxena and V.K. Saxena, 2008. Effects of supplemental chromium on interferon-gamma (IFN- γ) mRNA expression in response to Newcastle disease vaccine in broiler chicken. *Res. Vet. Sci.*, 85: 46-51. DOI: 10.1016/j.rvsc.2007.08.003.
- Burton, J.L., B.A. Mallard and D.N. Mowat, 1993. Effects of supplemental chromium on immune responses of periparturient and early lactation dairy cows. *J. Anim. Sci.*, 71: 1532-1539. <http://jas.fass.org/cgi/content/abstract/71/6/1532>.
- Chang, X. and D.N. Mowat, 1992. Supplemental chromium for stressed and growing feeder calves. *J. Anim. Sci.*, 70: 559-565. <http://jas.fass.org/cgi/content/abstract/70/2/559>.
- Debski, B., W. Zalewski, M.A. Gralak and T. Kosla, 2004. Chromium yeast supplementation of broilers in an industrial farming system. *J. Trace Element Med. Biol.*, 18: 47-51. DOI: 10.1016/j.jtemb.2004.02.003.
- Gross, W.B. and P.S. Siegel, 1983. Evaluation of heterophil to lymphocyte ratio as a measure of stress in chickens. *Avian Disease*, 27: 972-979. <http://avdi.allenpress.com/avdionline/?request=get-abstract&doi=10.1043>.
- Guo, Y.L., X.G. Luo, Z.L. Hao, B. Liu, J.L. Chen, F.S. Gao and S.X. Yu, 1999. Effect of chromium on growth performance, serum biochemical traits, immune function and carcass quality of broiler chickens. *Scientia Agric. Sinica.*, 32: 79-86. <http://www.ceps.com.tw/ec/ecjnlissuelist.aspx?jnlcattype=1&jnlptyp e=5&jnltype=43&newIssueIID=10284&jnliid=1004>.
- Jackson, A.R., S. Powell, S. Johnston, J.L. Shelton, T.D. Bidner, F.R. Valdez and L.L. Southern, 2008. The effect of chromium propionate on growth performance and carcass traits in broilers. *J. Appl. Poult. Res.*, 17: 476-481. <http://www.scopus.com/scopus/inward/record.url?eid=2-s2.0-57749172214&partnerID=40>.
- Khangarot, B.S., R.S. Rathore and D.M. Tripathi, 1999. Effects of chromium on humoral and cell-mediated immune responses and host resistance to disease in a freshwater catfish, *Saccobranchus fossilis* (Bloch). *Ecotoxicol. Environ. Safety*, 43: 11-20. DOI: 10.1006/eesa.1998.1722.
- Kim, Y.H., I.K. Han, Y.J. Choi, I.S. Shin, B.J. Chae and T.H. Kang, 1996. Effects of dietary levels of chromium picolinate on growth performance carcass quality and serum traits in broiler chicks. *Asian-Aust. J. Anim. Sci.*, 9: 341-347. <http://www.ajas.info/absView.asp>.
- Lee, D.N., F.Y. Wu, Y.H. Cheng, R.S. Lin and P.C. Wu, 2003. Effect of dietary chromium picolinate supplementation on growth performance and immune responses of broilers. *Asian-Aust. J. Anim. Sci.*, 16: 227-233. <http://www.ajas.info/absView.asp>.
- Lien, T.F., Y.M. Horng and K.H. Yang, 1999. Performance, serum characteristics, carcass traits and lipid metabolism of broilers as affected by supplement of chromium picolinate. *Br. Poult. Sci.*, 40: 357-363. DOI: 10.1080/00071669987458.
- Luo, X., Y.L. Guo, B. Liu, Z.L. Hao, J.L. Chen, F.S. Gao and S.X. Yu, 1999. Effect of dietary chromium on growth, serum biochemical traits and immune responses of broiler chicks during 0-3 weeks of age. *Acta Vet. Zootech. Sinica*, 30: 481-489. <http://www.xmsyxb.com/en/qkjs.asp>.
- McCarty, M.F., 1991. The case for supplemental chromium and a survey of clinical studies with chromium picolinate. *J. Appl. Nutr.*, 43: 58-66. <http://www.iaacn.org/JournalAppliedNutrition.cfm>.

- Moonsie-Shageer, S. and D.N. Mowat, 1993. Effect of level of supplemental chromium on performance, serum constituents and immuno status of stressed feeder calves. *J. Anim. Sci.*, 71: 232-238. <http://jas.fass.org/cgi/content/abstract/71/1/232>.
- Nam, K.T., H.Y. Lee, C.W. Kang and I.J. Yoo, 1995. The effects of dietary Chromium (Cr) in broiler chicks under heat stress. *Proceed. World Poultry Congress*, New Delhi, India, pp: 209-212.
- NRC (National Research Council), 1994. *Nutrient Requirements of Poultry*. Ninth Revised Edn. National Academy Press, Washington, DC.
- Ohba, H., Y. Suzuki and H. Ohba, 1986. Enhancement of ribonucleic acid synthesis by chromium (III)-bound chromatin. *J. Inorg. Biochem.*, 27: 179-188. DOI: 10.1016/0162-0134(86)80059-9.
- Sahin, K., N. Sahin, M. Onderci, F. Gursu and G. Cikim, 2002. Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities and some serum metabolites of broiler chickens. *Biol. Trace Element Res.*, 89: 53-64. ISI: 000178821300006.
- Samanta, S., S. Haldar and T.K. Ghosh, 2008. Production and carcass traits in broiler chickens given diets supplemented with inorganic trivalent chromium and an organic acid blend. *Br. Poultry Sci.*, 49: 155-163. DOI: 10.1080/00071660801946950.
- SAS Institute, 1997. *SAS Users Guide*. Version 6.12 Review Edn. SAS Institute Inc., Cary, NC.
- Schwartz, K. and W.A. Mertz, 1959. Chromium (III) and the glucose tolerance factor. *Arch. Biochem. Biophys.*, 85: 292-295. DOI: 10.1016/0003-9861(59)90479-5.
- Toghyani, M., M. Shivazad, A.A. Gheisari and S.H. Zarkesh, 2006. Performance, carcass traits and hematological parameters of heat-stressed broiler chicks in response to dietary levels of chromium picolinate. *Int. J. Poultry Sci.* 5 (1): 65-69. <http://www.pjbs.org/ijps/ab535.htm>.
- Toghyani, M., S. Zarkesh, M. Shivazad and A. Gheisari, 2007. Immune responses of broiler chicks fed chromium picolinate in heat stress condition. *J. Poultry Sci.*, 44: 330-334. <http://www.scopus.com/scopus/inward/record.url?eid=2-s2.0-34547600483&partnerID=40>.
- Uyanik, F., A. Atasever, S. Ozdmar and F. Aydin, 2002. Effects of dietary chromium chloride supplementation on performance, some serum parameters and immune response in broilers. *Biol. Trace Element Res.*, 90: 99-115. <http://www.ovid.com/site/catalog/Journal/1697.jsp>.
- Van De Ligt, J.L., M.D. Lindemann, R.J. Harmon, H.J. Monegue and G.L. Cromwell, 2002. Effect of chromium tripicolinate supplementation on porcine immune response during the postweaning period. *J. Anim. Sci.*, 80: 449-455. <http://jas.fass.org/cgi/content/abstract/80/2/449>.
- Vincent, J.B., 2000. The biochemistry of chromium. *J. Nutr.*, 130: 715-718. <http://jn.nutrition.org/cgi/content/abstract/130/4/715>.
- Wang, J.Y., D.T. Tsukayama, B.H. Wicklund and R.B. Gustilo, 1996. Inhibition of T and B cell proliferation by titanium, cobalt and chromium: Role of IL-2 and IL-6. *J. Biomed. Mater. Res.*, 32: 655-661. <http://www.interscience.wiley.com/journal/96516924>.